Preliminary Communication

INHIBITORY EFFECT OF TNFα ANTIBODIES ON SYNOVIAL CELL INTERLEUKIN-1 PRODUCTION IN RHEUMATOID ARTHRITIS

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Summary The effect of tumour necrosis factor (TNFα) antibodies on synovial cell interleukin-1 (IL-1) production was investigated in 7 patients with rheumatoid arthritis and in 7 with osteoarthritis. Synovial cell IL-1 production was significantly reduced by anti-TNFα antibody in cultures from patients with rheumatoid arthritis, but anti-lymphotoxin antibody did not have this effect (except in 1 culture). In cultures from patients with osteoarthritis spontaneous IL-1 production was low, despite high concentrations of TNFα, and IL-1 production was not inhibited by anti-TNFα antibody. In rheumatoid arthritis, TNFα may be the main inducer of IL-1, and anti-TNFα agents may be useful in treatment.

INTRODUCTION

RHEUMATOID arthritis is a common inflammatory disease in which chronic activation of the resident and infiltrating synovial cells causes destruction of cartilage and bone and leads to fibrosis.[1] The autoimmune mechanisms that induce joint damage remain unclear, but the cytokines interleukin-1 (IL-1)[2] and tumour necrosis factor (TNFα)[3] have been found in the synovial fluid of patients with rheumatoid arthritis. These cytokines are potent inducers of both cartilage[4,5] and bone[6,7] destruction and, in rabbits, intra-articular IL-1 can induce arthritis.[8] We have used cDNA probes to survey cytokine synthesis in synovial cells from patients with rheumatoid arthritis, and have detected mRNA for T-cell-derived mediators such as interleukin-2 (IL-2), interferon γ (IFNγ),[9] and lymphotoxin, and for macrophage-derived mediators such as IL-1α, IL-1β, TNFα,[10] and IL-6.[11] Although these and other cytokines may contribute to persistence of the inflammatory processes that occur in rheumatoid joints, their relative importance remains unclear.

H. GURNEY AND OTHERS: REFERENCES—*continued*

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Cultures of mononuclear cells extracted from the synovium or synovial fluid of rheumatoid joints produce cytokines for up to 6 days without extrinsic stimulation.[9] This observation indicates that the stimulus (or stimuli) for cytokine production could be isolated from the cultures. IL-1 production can be induced by IL-1 itself,[12] TNFα,[13] lymphotoxin, and immune complexes.[14] We have investigated the role of TNFα and lymphotoxin (which have overlapping biological functions[15]) in the regulation of IL-1 production in rheumatoid arthritis compared with osteoarthritis.

PATIENTS AND METHODS

We examined 7 patients with "classical" rheumatoid arthritis as defined by the revised American Rheumatism Association criteria,[16] and 7 patients with osteoarthritis (see table I for patient details). Mononuclear cells were extracted either from synovial fluid samples aspirated from joints during routine treatment, or from synovial membrane samples obtained during total knee or hip replacement. These cells, referred to as synovial cells in this report, were a heterogeneous mixture of all the infiltrating mononuclear cells and connective tissue cells found in synovial joints.

Synovial fluid was collected in heparinised bottles and separated by 'Ficoll-Hypaque' (specific density 1·077 g/ml) density gradient centrifugation. Synovial membrane tissue was digested in Roswell Park Memorial Institute (RPMI) medium that contained 5% fetal calf serum, 5 mg/ml collagenase type IV (Sigma), and 0·15 mg/ml DNAse type I (Sigma), and incubated at 37°C for 4 h. After incubation, the tissue was pipetted through a 200 μl nylon mesh into a sterile beaker. The cells were then washed 3 times in complete medium (RPMI 1640 with 10% fetal calf serum). Synovial membrane or synovial fluid cells were cultured at 1×10^6 cells/ml in 2 ml of complete medium in 24-well plates (Falcon 3047) without exogenous stimulus for up to 6 days. Cells were incubated with antibodies sufficient to neutralise 500 units (10 000 pg/ml) of TNFα (rabbit anti-TNFα antibody serum, Genentech), 500 units (5000 pg/ml) of lymphotoxin (rabbit anti-lymphotoxin antibody serum, Genentech), or an equivalent amount of control rabbit IgG (gift of Dr G. Howells). Cells and supernatants were harvested after 1, 3, and 6 days in culture. In the earlier experiments, control (untreated) cultures were also included to exclude the possibility that a contaminant of rabbit immunoglobulin stimulated the synovial cells, but this was not the case and untreated cultures were omitted in later experiments.

TNFα and lymphotoxin protein were measured by enzyme-linked immunosorbent assay (ELISA) of the supernatants in duplicate as previously described.[3,17] (Polyclonal and monoclonal anti-TNFα reagents used in the TNFα ELISA were a kind gift of Dr M. Shepard [Genentech]; polyclonal and monoclonal anti-lymphotoxin reagents used in the lymphotoxin ELISA were kindly provided by Dr T. Meager [NIBSC].) Results are expressed as pg/ml of TNFα or lymphotoxin derived from a standard curve for recombinant TNFα and lymphotoxin protein (Genentech). The lower limit of sensitivity of the ELISA was 50 pg/ml for both TNFα and lymphotoxin. IL-1 concentrations were determined by the mouse thymocyte assay of triplicate samples as previously described.[14] Thymidine incorporation was converted into units of IL-1/ml from the recombinant IL-1β standard curve. IL-1 specificity of the thymocyte assay was checked by pre-incubation of day 1 control supernatants with antibodies to IL-1α, IL-1β, and IL-6, and was also confirmed in day 3 and day 6 culture supernatants. No other human cytokines are known to stimulate mouse thymocytes.

The cellular composition of synovial samples was determined by 2-colour immunofluorescence and flow cytometry. For each analysis 5×10^5 cells were incubated for 30 min at 4°C with anti-CD3 (Leu-4 fluorescein isothiocyanate [FITC], Becton Dickinson), anti-HLA DR (DR-PE, Becton Dickinson), or anti-CD14 (Leu M3-FITC, Becton Dickinson). Cells were then washed 3 times in buffer and analysed with a fluorescence activated cell sorter flow cytometer (Becton Dickinson). Results were expressed as the percentage of total synovial cell population which

TABLE I—TNFα, LYMPHOTOXIN (LT), AND IL-1 PRODUCTION BY SYNOVIAL CELLS AFTER 1 DAY IN CULTURE

Patient details*	TNFα (pg/ml)	LT (pg/ml)	IL-1 (U/ml)	Cellular composition (%) CD3+ (CD3+/DR+)	Cellular composition (%) CD14+
Rheumatoid arthritis					
1: 74, F; N, P	60	<50	4	NA	NA
2: 64, M; P	2000	<50	18	62 (35)	7
3: 65, M; P, M	580	<50	10	NA	NA
4: 43, F; G, N	152	<50	3	36 (64)	35
5: 66, M; A, P	82	<50	4	69 (60)	31
6: 88, F; P	100	<50	15	65 (56)	21
7: 56, M; N	300	<50	†	68 (42)	26
Osteoarthritis					
1: 73, F; N	1600	<50	0·8	7 (33)	23
2: 79, F; N	1970	<50	1·0	12 (40)	40
3: 59, F; N	70	<50	6·0	32 (40)	17
4: 69, F; N	920	<50	<0·2	11 (35)	20
5: 70, M; ..	263	<50	0·9	34 (30)	21
6: 68, F; ..	50	<50	<0·1	20 (32)	22
7: 64, F; N	780	<50	0·2	15 (28)	40

*Shown as patient number: age (yr), sex; drug treatment (N = non-steroidal anti-inflammatory drug; P = prednisolone; M = methotrexate; G = gold; and A = azathioprine).
†IL-1 units not assessed as thymocyte proliferation not completely inhibited with neutralising antibodies to IL-1α and IL-1β.
NA = not available due to insufficient tissue in specimen.

were T cells (CD3-positive [CD3+]) or macrophages/monocytes (CD14+). In addition the proportion of activated T cells was identified as those T cells which were also class II+ (CD3+/DR+).

RESULTS

TNFα, lymphotoxin, and IL-1 concentrations produced by the synovial cultures after 1 day in culture are summarised in table I. TNFα protein was detected in all 7 rheumatoid synovial cell cultures incubated with rabbit IgG, at concentrations of 60–2000 pg/ml. In rheumatoid arthritis cultures incubated with polyclonal anti-TNFα antibody, TNFα (at 50 pg/ml or above) was not detected: neutralising antibody efficiently removed secreted protein, whereas no effect on TNFα concentrations was observed in cultures incubated with polyclonal anti-lymphotoxin antibody (results not shown) which confirms that the anti-lymphotoxin serum used does not cross-react with TNFα.[18] Lymphotoxin protein was not detected in any of the supernatants, which indicated lymphotoxin concentrations below the 50 pg/ml lower limit of sensitivity of the ELISA. Thymocyte proliferation induced by rheumatoid arthritis supernatants (with the exception of patient 7) was completely inhibited by neutralising antibodies to IL-1α and IL-1β, but not by neutralising antibodies to IL-6. From the standard curve derived from recombinant human IL-1β, the concentrations of IL-1 in the supernatants were determined and ranged from 3 to 18 U/ml—well above 1 U/ml, the concentration known to induce half-maximum proliferation of thymocytes,[10] and which can induce cartilage and bone destruction in vitro. The patients with rheumatoid arthritis were on various treatments, and all but one was on second-line therapy due to the disease severity. Nevertheless, these treatments did not prevent TNFα or IL-1 production, although they may account for the undetectable levels of lymphotoxin and may have reduced TNFα and IL-1 concentrations. T cells (CD3+) were the predominant cell type in rheumatoid synovial joint cultures, and a large proportion were activated (HLA-DR+); cells of monocytic/macrophage lineage (CD14+) were also common. Synovial cell cultures from patients with osteoarthritis also had high levels of TNFα (50–1970 pg/ml), and lymphotoxin protein (at a concentration above 50 pg/ml) was not detected (table I). The high TNFα concentrations indicate that inflammatory cytokines and cells are involved in osteoarthritis, and that the disease is not just due to mechanical wear and tear. However, in contrast to rheumatoid synovial cells (with the exception of patient 3) IL-1 concentrations were very low. The cellular composition of the osteoarthritis cultures differed from that of rheumatoid arthritis cultures: the proportion of infiltrating T lymphocytes was significantly smaller, the proportion of CD14+ cells (monocytes/macrophages) was similar to the rheumatoid arthritis cultures, but the largest proportion of cells (presumably fibroblasts) were not identified with lymphocytic or macrophage markers (table I).

The effect on IL-1 production of neutralising antibodies to TNFα and lymphotoxin is summarised in table II for rheumatoid arthritis and osteoarthritis synovial cell cultures. The percentage inhibition of IL-1 production, compared with control rabbit IgG culture, is only assigned a value where the mean IL-1 concentration (assayed in triplicate) was significantly different from that obtained in the control culture (data not shown). In all day 3 rheumatoid cultures anti-TNFα antibody significantly inhibited IL-1 bioactivity (50–99%), whereas little effect on IL-1 concentrations was observed at day 1: therefore the antibody may inhibit restimulation of the synovial cells but allow existing synthesis to be completed. This effect was not due to any toxicity of the rabbit anti-TNFα antibody (data not shown). Fig 1 shows a typical result for a rheumatoid cell

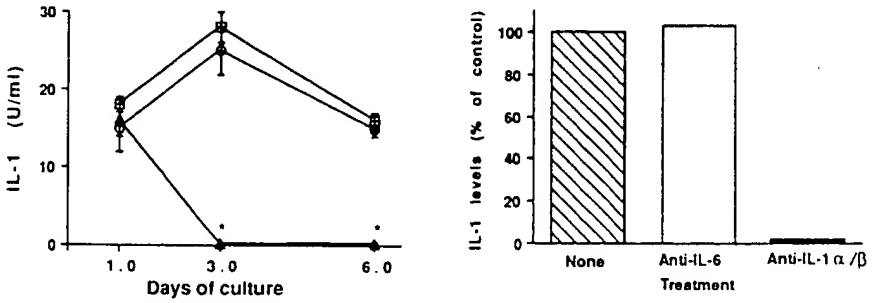

Fig 1—Culture of rheumatoid arthritis synovial membrane cells with polyclonal antibodies to TNFα (▲), polyclonal antibodies to lymphotoxin (△), or equivalent amount of control rabbit IgG (_).

Specificity of the assay for IL-1 shown by preincubation of day 1 control supernatants with neutralising antibodies specific for IL-1 α,β or IL-6.
*$p < 0.001$ (Student's t test).

TABLE II—EFFECT OF ANTI-TNFα ANTIBODY AND ANTI-LYMPHOTOXIN ANTIBODY ON IL-1 PRODUCTION BY SYNOVIAL CELL CULTURES

	Inhibition of IL-1 production (%)					
	Rabbit anti-TNFα antibody			Rabbit anti-lymphotoxin antibody		
Patient	Day 1	Day 3	Day 6	Day 1	Day 3	Day 6
Rheumatoid arthritis						
1	38	50	NI	NI	NI	NI
2	NI	99	99	NI	NI	NI
3	NI	98	97	NI	NI	NI
4	NI	90	96	NI	NI	NI
5	90	99	90	NI	92	60
6	NI	64	64	NI	NI	NI
7	NI	66	NI	NI	NI	NI
Osteoarthritis						
1	NI	NI	NI	NI	NI	NI
2	NI	NI	NI	NI	NI	NI
3	NI	98	97	NI	NI	NI
4	NI	NI	NI	NI	NI	NI
5	NI	NI	NI	NI	NI	NI
6	NI	NI	NI	NI	NI	NI
7	NI	NI	NI	NI	NI	NI

Results presented as % inhibition of IL-1 bioactivity by neutralising anti-TNFα antibody and anti-lymphotoxin antibody, compared with control culture (rabbit IgG).
NI = no inhibition (ie, no significant difference from mean IL-1 concentration in control culture).

culture. Inhibition of IL-1 activity was still evident after 6 days of culture except for patients 1 and 7, in whom there was no IL-1 in the control cultures and hence no apparent inhibition by TNFα. By contrast, no significant inhibition was observed with anti-lymphotoxin antibody except for the culture from patient 5, in which 92% of IL-1 bioactivity was inhibited at day 3 by anti-lymphotoxin antibody: this finding indicates that some lymphotoxin, below the assay limit of 50 pg/ml, was present in this culture sufficient to induce IL-1, possibly in synergy with other mediators. No significant effect on IL-1 concentrations was observed in the other 6 rheumatoid arthritis cultures incubated with anti-lymphotoxin antibody. For osteoarthritis synovial cell cultures no effect on IL-1 levels was observed (table II): fig 2 shows a representative result (from patient 1). The IL-1 concentration for patient 3 was in the range observed for rheumatoid arthritis cultures (6 U/ml), but unlike the other 6 osteoarthritis cultures IL-1 concentrations were maintained in culture and could be inhibited (98% day 3, 97% day 6) by anti-TNFα antibody.

DISCUSSION

The apparent dissociation of TNFα and IL-1 production may indicate that other factors are involved in IL-1 induction and that these molecules are found in the rheumatoid arthritis but not the osteoarthritis synovial cultures. We have shown, for example, that immune complexes induce IL-1 production,[14] and are found in large quantities in rheumatoid but not osteoarthritic joints.[19] Similarly, other cytokines induce IL-1 synthesis, such as granulocyte-macrophage colony stimulating factor (GM-CSF),[20] and others such as interferon γ synergise with TNFα to induce IL-1:[21] we have detected both of these cytokines in rheumatoid arthritis synovial cells. Immune-complex induction of IL-1 can also be enhanced by interferon γ and by TNFα.[14] It is also possible that osteoarthritis synovial cultures produce an inhibitor that inactivates TNFα.[22]

However, our findings indicate that TNFα is the dominant inducer of IL-1 production in rheumatoid but not in osteoarthritic joints: although other cytokines or agents (eg, immune complexes) may synergise with TNFα to induce IL-1, only anti-TNFα antibody prevents IL-1 production in rheumatoid joints. TNFα may damage rheumatoid joints both directly and indirectly by IL-1 induction, and may therefore be an important target for therapy in rheumatoid arthritis. Neutralising antibodies to TNFα have been used successfully to protect against TNFα effects in lethal bacteraemia in baboons[23] and cerebral malaria in mice.[24] Antibodies to TNFα injected locally into a rheumatoid joint may be a useful therapy in severe rheumatoid arthritis and may help to restore the immunological balance, and we have preliminary evidence that monoclonal anti-TNFα antibodies can also inhibit IL-1 production in these rheumatoid arthritis synovial cultures (unpublished observations). Alternatively, drug therapy to inhibit TNFα production or to neutralise its effects may help to control the inflammatory process in rheumatoid arthritis.

The precise mechanism whereby rabbit anti-TNFα inhibits IL-1 production in the rheumatoid arthritis cultures is not known, although we have evidence that it also inhibits IL-1 mRNA production (data not shown), which indicates that the inhibition is at induction rather than by an effect on IL-1 secretion or processing. The differential effect of anti-TNFα on the rheumatoid arthritis and osteoarthritis synovial cell production of IL-1 is not fully understood, but our results indicate that inhibition of IL-1 activity by anti-TNFα in culture was only apparent with a high initial

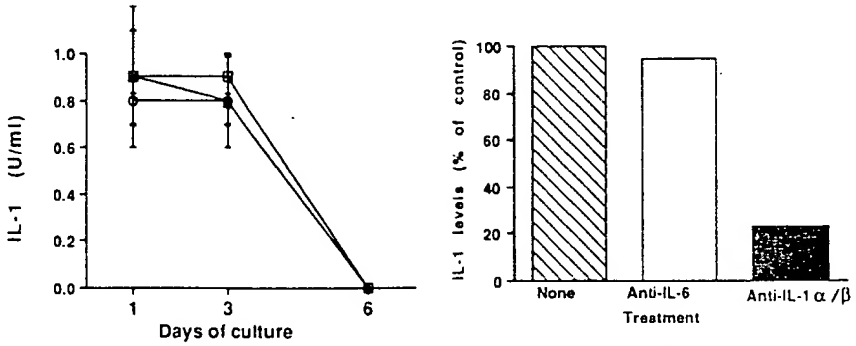

Fig 2—Culture of osteoarthritis synovial membrane cells with polyclonal antibodies to TNFα (▲), polyclonal antibodies to lymphotoxin ( ), or equivalent amount of control rabbit IgG ( ).

Specificity of IL-1 assay shown as for fig 1.

IL-1 concentration. In most osteoarthritis synovial cultures the initial IL-1 concentration was low and declined rapidly in culture, so this process would not be further accelerated by anti-TNFα. However, the reason for high IL-1 concentrations in rheumatoid arthritis synovial cultures but not in osteoarthritis synovial cultures, despite the presence of high concentrations of TNFα in both diseases, remains unclear. From the cellular composition and previous studies it is likely (but not proven) that macrophages make most of the TNFα and IL-1. If true, activated macrophages in osteoarthritis may selectively produce TNFα, but not other cytokines such as IL-1.

We thank Dr H. M. Shepard, Dr T. Chen, and Dr T. Meager for the TNFα and lymphotoxin ELISA reagents; Dr L. Aarden for neutralising anti IL-6 antibody; Dr S. Gillis and Dr C. Henney for neutralising anti-IL-1α and IL-1β reagents; Ms J. Ellis for organising the tissue samples; Ms K. Hartley for processing the tissue; Dr M. Kahan for FACS analysis; and Mr M. Turner for helpful comments during preparation of this manuscript. This work was supported by the Arthritis and Rheumatism Council and the Sunley Trust.

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Reviews of Books

Endovaginal Ultrasound

Steven R. Goldstein. New York: Alan R. Liss. 1989. Pp 175. $55. ISBN 0-845142631.

HAPPINESS is not having an uncomfortably full bladder. Unfortunately a full bladder is necessary for conventional ultrasound examination early in pregnancy, and sometimes as late as the twentieth week if the fetus is playing hard to see. The joy of identifying her baby only a few weeks after she has missed a period has, for many a mother, been a rapture modified by sensations that she is going to lose control. Now changes are afoot. Vaginal ultrasound does not need a full bladder; indeed, with this approach such fullness may obscure or distort pelvic anatomy. Vaginal ultrasound also eliminates poor visualisation associated with an excessively fat abdominal wall. The image it produces is very clear owing to excellent resolution by the equipment, the only drawback being a limited field of view. The probes used may be somewhat forbidding at first sight, but they are well tolerated by most women, especially by those who have experienced abdominal scans. The information that can be obtained in the first trimester by skilled operators is unapproachable by any other method of imaging. Ectopic pregnancies can be identifed and differentiated from other adnexal swellings; fetal anatomy can be clarified at 8–10 weeks in considerable detail, although the spine and other organs still require examination at 17–20 weeks for complete certainty; and failing pregnancies within the uterus can be identified. In patients with a history of recurrent abortion cervical incompetence can be assessed and the effect of cerclage monitored. Abnormalities of pregnancy are only part of the information available from vaginal ultrasound. Salpingitis and small pelvic tumours can be diagnosed and the technique is also a very real advance in assisted reproduction since follicular growth and endometrial thickness can be measured and oocyte retrieval simplified.

The practising enthusiasts who have written *Endovaginal Ultrasound* convey all of this information in their excellent primer. They clearly describe the relation of the image to pelvic anatomy, which is not always obvious to the novitiate. The illustrations are also clear and informative. Techniques are described succinctly but are not simplified. The book concludes with axioms that are worth contemplation both by beginners and by more experienced practitioners. Those who wish to inquire about the value of vaginal ultrasound, or to learn the technique, would do well to consult this book; if they follow up the carefully chosen references they will expand their new-found understanding.

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Manual of Critical Care Procedures

Edited by Lyle D. Victor. Dearborn: Aspen. 1989. Pp 215. $67. ISBN 0-834200341.

ENTRANTS to the specialty of intensive care come from diverse primary training backgrounds, so they often lack some of the necessary practical skills. The contributors to this manual, which is a compilation of procedures commonly carried out in the editor's cardiorespiratory